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Efficient Peptide Coupling Involving Sterically Hindered Amino Acids

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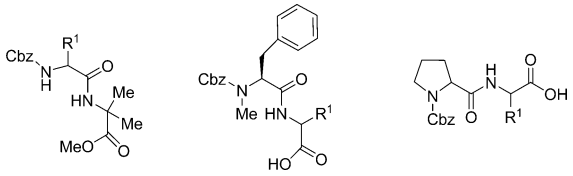

Hindered amino acids have been introduced into peptide chains by coupling *N*-(Cbz- and Fmoc-α-aminoacyl)benzotriazoles with amino acids, wherein at least one of the components was sterically hindered, to provide compounds **3a−e, (3c +3 c′), 5a−d, (5a + 5a′), 6a−c, (6b + 6b′), 8a−c, 9a−e, 10a−d,** and **(10a + 10a′)** in isolated yields of 41−95% with complete retention of chirality as evidenced by NMR and HPLC analysis. The benzotriazole activation methodology is a new route for the synthesis of sterically hindered peptides. (Note: compound numbers written within brackets represent diastereomeric mixtures or racemates; compound numbers without brackets represent enantiomers.)

Introduction

Sterically hindered amino acids, such as Aib (2-methylalanine), Iva (2-ethylalanine), and Deg (2,2,-diethylglycine) (Figure 1) are constituents of naturally occurring peptaibols,[1−4] a large group of peptide antibiotics isolated from soil fungi.

The presence of an extra alkyl group at C^{α} significantly restricts the accessible conformational space in α,α-disubstituted amino acids; this forces the chain to bend in peptides containing these sterically hindered amino acids.[5−7] Thus, peptides incorporating α,α-disubstituted amino acids, such as Aib or Iva, have rigid backbones through the formation of helices and *β*-turns.[8] These conformational properties of α,α-disubstituted amino acids confer interesting biological activity to peptaibols, which can form voltage dependent ion channels in bilayer cellular membranes and can cause, at high concentration, cell lysis.[2] These properties make α,α-disubstituted amino acids important for peptide and medicinal chemistry. Thus, Aib, Iva, and Deg are often used to study the relationships existing between structure, stability, and function in bioactive peptides.[9] However, the reactivities of both (i) amino groups and (ii) activated carboxyl groups of sterically hindered α,α-disubstituted amino acids are significantly lower than those of typical α-amino acids; hence their incorporation even into short peptides has been challenging.[10]

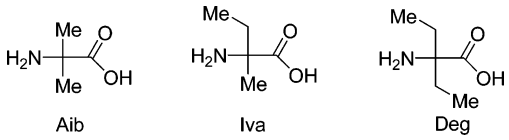

FIGURE 1. Sterically hindered amino acids.

N-Substitution at the peptide bond also induces steric modification and mimics various biological environments. N-Substituted peptides are important constituents of a number of naturally occurring peptides like cyclosporines,[11] didemnins[12]

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(and references therein), and dolastatins[13] (and references therein). *N*-Methyl peptides exhibit antibiotic,[14] anticancer,[15−17] and antiviral activity.[18]

The cyclic side chain of the naturally occurring N-alkylated amino acid proline locks a peptide backbone at a dihedral angle of approximately −75°. Thus proline has exceptional conformational rigidity compared to other amino acids and consequently is often found in very tight turns in protein structures. Proline also introduces *kinks* into α helices, since it is unable to adopt a normal helical conformation. These conformational properties of proline and proline rich sequences exhibit interesting biological and medicinal roles: (1) drug delivery activity,[19] (2) important components of collagen,[19] (3) intracellular cell signaling, and (4) quenching reactive oxygen species.[20]

Previously, we reported the successful preparation of peptides without detectable racemization (<5% by NMR analysis and <1% by HPLC analysis) in average yields of 88%.[21−25] The present paper reports convenient procedures for the incorporation of hindered amino acids into peptide chains.

Results and Discussions

I. Preparation of Aib-Containing Dipeptides. (a) Using Carboxyl Activated N-Protected Aib. We now show that amino acids **2a**−**c** and **(2c + 2c′)** (compound numbers written within brackets represent diastereomeric mixtures or racemates; compound numbers without brackets represent enantiomers) couple with *N*-(Cbz- and Fmoc-Aib-)Bt **1a,b** (Bt = benzotriazol-1-yl) in partially aqueous solution (CH_3CN/H_2O) in the presence of Et_3N in 1 h at 20 °C to give dipeptides **3a**−**e** and **(3c + 3c′)** (67−92%) isolated without chromatography (Scheme 1 and Table 1). The enantiopurity of the dipeptides **3a**−**e** was supported by HPLC analyses using a Chirobiotic T column (detection at 254 nm, flow rate 0.5 mL/min, and MeOH as solvent). As expected, HPLC analysis of the enantiopure dipeptides **3a**−**e** showed a single peak. In contrast, two peaks of equal intensity were observed for the corresponding racemic mixture **(3c + 3c′)** (Table 1).

SCHEME 1

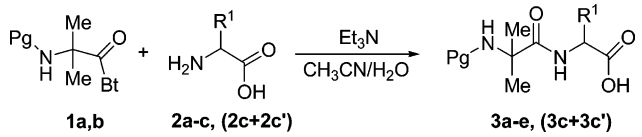

TABLE 1. Preparation of N-Terminus Aib Dipeptides 3a−e and (3c + 3c′) from *N*-(Cbz- and Fmoc-Aib-)Bt 1a,b and Free Amino Acids 2a−c and (2c + 2c′)

entry	amino acid	product	yield (%)[a]	retention time (min)
1	L-Phe-OH **2a**	Cbz-Aib-L-Phe-OH **3a**	84	6.19
2	L-Trp-OH **2b**	Cbz-Aib-L-Trp-OH **3b**	85	5.79
3	L-Met-OH **2c**	Cbz-Aib-L-Met-OH **3c**	91	6.43
4	DL-Met (**2c + 2c′**)	Cbz-Aib-DL-Met-OH (**3c + 3c′**)	92	6.40, 7.34
5	L-Phe-OH **2a**	Fmoc-Aib-L-Phe-OH **3d**	67	5.39
6	L-Met-OH **2c**	Fmoc-Aib-L-Met-OH **3e**	70	5.82

[a] Isolated yield.

Recent literature approaches to realize coupling at the carbonyl group of Aib to give hindered peptides (Scheme 2) include (i) the use of DCC or DCC/HOBT[26] (and references within), (ii) 2-mercaptopyridone-1-oxide based uronium salts (HOTT and TOTT),[27] (iii) the active ester coupling method,[28] (iv) CIP (2-chloro-1,3-dimethylimidazolidium hexaflurophosphate) in the presence of additives such as HOAt or HODhbt,[29,30] (v) enzymatic coupling,[31] (vi) PyBOP and PyBroP,[26] and (vii) polymer-supported BOP coupling reagents.[32] The methods of Scheme 2 give Aib-containing dipeptides in variable yields (23−96%) depending upon the protecting group (Pg) and −R group incorporated. Most need labor intensive procedures (ref 26 and references therein), utilize expensive or unstable reagents,[31] and/or need an additional deprotection[28] step, because amino acid esters are used.[26−29,32] In comparison, our methodology offers simple preparative and workup procedures, takes less time to complete, uses inexpensive reagents, gives high yields, and frequently allows free amino acids as coupling components.

(b) By Coupling at the Amino Group of Aib. Our attempts under varying conditions (temperature, solvent systems, time) and reagents (base, additives) to couple *N*-(protected-α-aminoacyl)benzotriazoles with the NH_2 of free Aib (i.e., $H_2NMe_2CCO_2H$), resulted in extensive hydrolysis of **1c**−**f** and **(1c + 1c′)**. However, Aib methyl ester **4** was successfully coupled in the presence of Et_3N (in acetonitrile) at 20 °C in 24−36 h (Scheme 3 and Table 2). Under microwave irradiation in the presence of 1 equiv of Et_3N in THF as solvent at 55 and 60 °C (Scheme 3 and Table 2) the preparation of compounds **5a**−**d** and **(5a + 5a′)** needed 1 h. Under conventional heating

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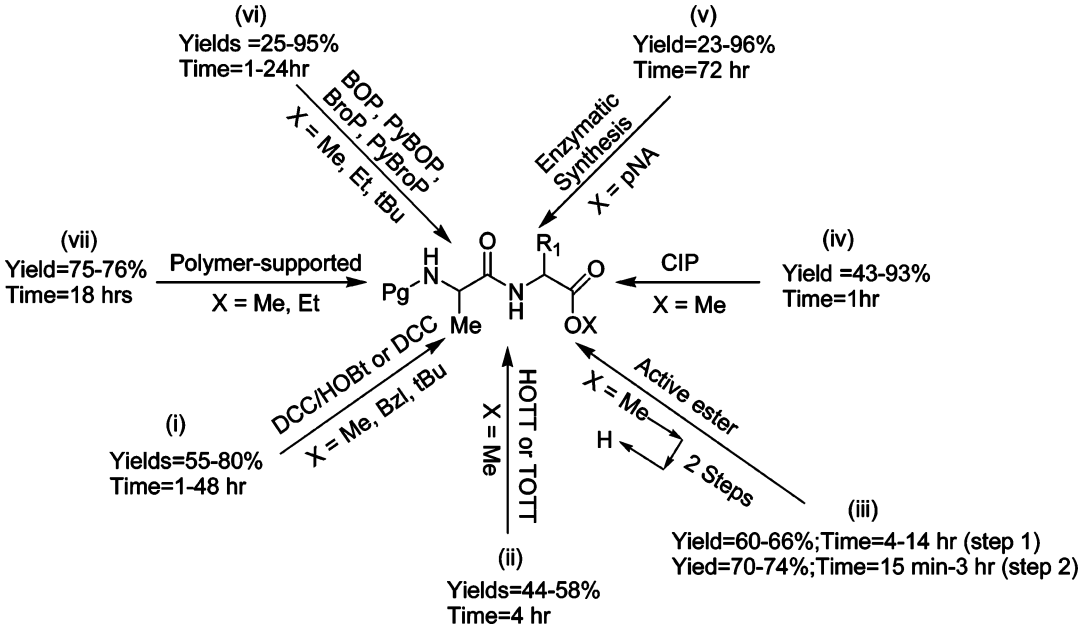
SCHEME 2. Literature Peptide Coupling Procedures for Activating the Carboxyl Group of Aib



SCHEME 3

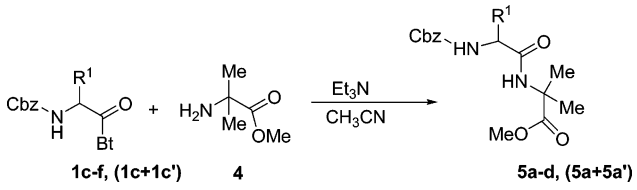


TABLE 2. Preparation of C-Terminus Aib Dipeptides 5a−d and (5a + 5a′) from *N*-(Cbz-α-aminoacyl) Benzotriazoles 1c−f, (1c + 1c′)

			yield (%)[a]	
entry	reactant	product	20 °C[b]	MW[c]
1	Cbz-L-Phe-Bt **1c**	Cbz-L-Phe-Aib-OMe **5a**[d]	41	72
2	Cbz-DL-Phe-Bt (**1c** + **1c′**)	Cbz-DL-Phe-Aib-OMe (**5a** + **5a′**)[e]	41	71
3	Cbz-L-Met-Bt **1d**	Cbz-L-Met-Aib-OMe **5b**[f]	78	79
4	Cbz-L-Trp-Bt **1e**	Cbz-L-Trp-Aib-OMe **5c**	41	80
5	Cbz-Gly-Bt **1f**	Cbz-Gly-Aib-OMe **5d**	64	79

[a] Isolated yield. [b] Reaction time = 24−36 h. [c] Power 200 W for 30 min at 55 °C and 300 W for 30 min at 60 °C. [d] HPLC for **5a**: 6.35 min. [e] HPLC for **(5a + 5a′)**: 6.43 and 7.17 min. [f] HPLC for **5b**: 7.17 min.

conditions at 60 °C lower yields and longer reactions times in comparison to those obtained with microwave technique were obtained.

Analysis by HPLC (Table 2) of the compounds **5a,b** and **(5a + 5a′),** prepared by the MW technique, confirmed the absence of racemization. Single peaks were obtained for the enantiomers **5a** and **5b** at 6.35 and 7.17 min, respectively, whereas the racemate **(5a + 5a′)** gave two peaks at 6.43 and 7.17 min.

Previous couplings at the NH group of Aib (Scheme 4) utilized (i) the active ester method,[33] (ii) polymer-supported coupling,[34] (iii) coupling reagent HOTT,[27] (iv) coupling reagent TOTT,[27] (v) 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT),[35] and (vi) 2-chloro-4,6-[(heptadecaflurocarbonyl)oxy]-1,3,5-triazine (^{F}CDMT).[36] The literature methods of Scheme 4 led to dipeptides in moderate to high yields (35−97%) depending upon the Pg and −R group incorporated. Reaction times ranged from 4 to 20 h. Some involved low temperatures or two-step procedures.[35,36] Our methodology provides yields of 71−80% in 1 h with convenient preparative procedures, simple workup procedures (the byproduct, BtH, is easily dissolved using dilute acids or bases), and insensitivity to water.

II. Preparation of Dipeptides Containing an *N*-Methyl Amino Acid. (a) From a Carboxyl Activated N-Protected *N*-Methyl Amino Acid. Amino acids **2c−e** and **(2d + 2d′)** were coupled successfully with Cbz-L-*N*(Me)-Phe-Bt **1g** in partially aqueous solution (CH_3CN/H_2O) in the presence of Et_3N during 1 h at room temperature (Scheme 5 and Table 3). After washing with 4 N HCl, pure dipeptides **6a−c** and **(6b + 6b′)** were obtained in yields ranging of 72−93%.

Variable temperature ^{1}H NMR confirmed that the complex peaks obtained for **6a−c** at 20 °C were due to restricted rotation at the amide bonds (Figure 2). For example, the expected doublet arising from the methyl protons of the L-Ala fragment in **6b** collapsed to an apparent triplet at 25 °C which was subsequently resolved at 100 °C into a clean doublet, with improved resolution, demonstrating the absence of racemization (Figure 2). Similar behavior was shown by **6b,c** and **(6b + 6b′)**. HPLC analysis (Table 3) of the enantiopure LL-dipeptides **6a−c** showed for each a single peak. In contrast, two peaks of equal intensity were observed for the corresponding diastereomeric mixture **(6b + 6b′)**.

Literature methods for the synthesis of peptides incorporating N-alkylated amino acids (Scheme 6) use the usual carbodiimide based coupling reagents including (i) DCCI-HOBt,[37] (ii) DCCI-HONSu,[37] and (iii) EDC-HOBt.[38] Additional methods utilize

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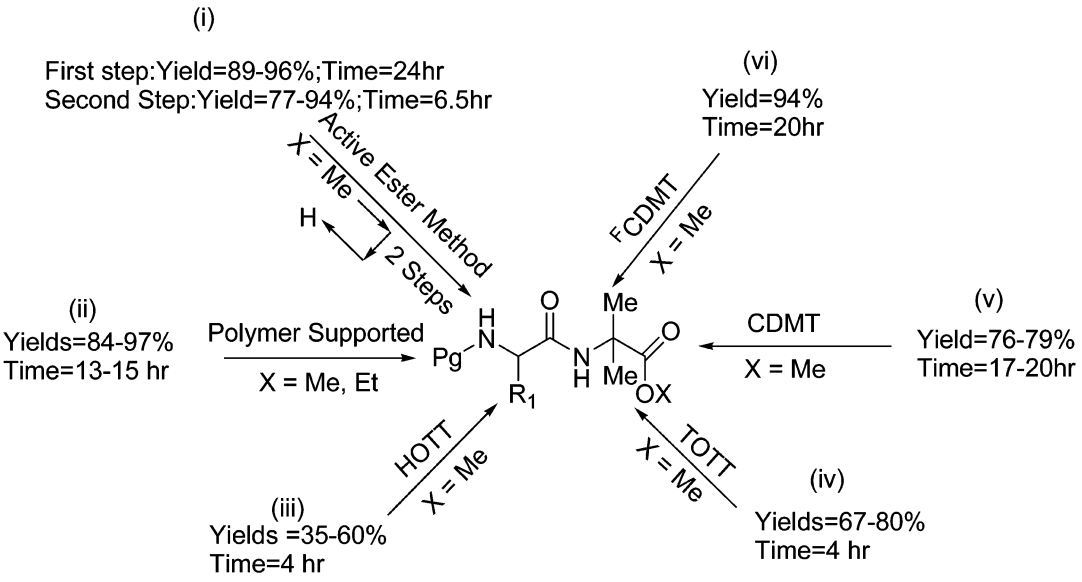
SCHEME 4. Literature Peptide Coupling Procedures with Amino Group of Aib



SCHEME 5

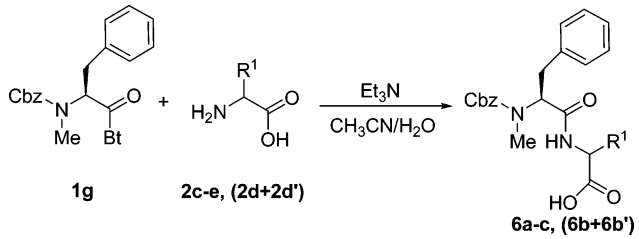


TABLE 3. Preparation of Dipeptides 6a−c and (6b + 6b′) from Cbz-L-*N*(Me)-Phe-Bt 1g and Free Amino Acids 2c−e and (2d + 2d′)

entry	reactant	product	yield (%)[a]	retention time (min)
1	L-Met-OH **2c**	Cbz-L-*N*(Me)-Phe-L-Met-OH **6a**	93	7.17
2	L-Ala-OH **2d**	Cbz-L-*N*(Me)-Phe-L-Ala-OH **6b**	72	6.56
3	DL-Ala-OH (**2d** + **2d′**)	Cbz-L-*N*(Me)-Phe-DL-Ala-OH (**6b** + **6b′**)	72	6.60, 7.16
4	Gly-OH **2e**	Cbz-L-*N*(Me)-Phe-Gly-OH **6c**	82	7.34

[a] Isolated yield.

(iv) a three-step procedure via oxazolidinones,[39] and (v) N-methylation with diazomethane after coupling of amino acid chlorides with amino acid methyl esters.[40] Reported yields range from 40 to 96% depending upon the Pg and −R group incorporated. The two-step procedure involving toxic diazomethane has a reaction time of 1.5 h.[40] Other reaction times in Scheme 6 vary from 12 to 48 h.[37−39] Davis et al. observed racemization (10−35%) during the coupling of benzoyl-*N*-alkylated amino acids with amino acid methyl esters.[37] Our one-step methodology provides optically pure dipeptides in yields of 72−93% using simple preparative and workup procedures in reaction times of 1 h.

(b) By Coupling at the NH Group of Free *N*-methyl Amino Acids. We adopted procedures similar to those of section **I(b)** in the Results and Discussion to couple Cbz-(aminoacyl)Bt **1c−d,f** with the NH group of *N*-methyl amino acid ester **7a**. Dipeptides **8a−c** were thus prepared (Scheme 7 and Table 4) in 72−80% yield during 1 h using microwave irradiation (for conditions see Table 4). Without microwave irradiation the reactions needed 12−18 h.

The room-temperature ^{1}H NMR showed the existence of rotamers for **8a−c**. The results obtained from high-temperature NMR were difficult to interpret precisely; all peaks appeared as broadened singlets because of the low solubility of compounds **8a−c** in DMSO. However, the elemental analyses of **8a−c** confirmed their formation, and HPLC analyses of **8a,b** revealed single retention times for each, supporting their enantiopurity (Table 4).

Additionally, the structure of compound **8b** was confirmed by the 2D homonuclear correlations COSY and NOESY. The COSY spectrum presented (see Figure 3 in Supporting Information) confirmed the sequence of the amino acids in peptide **8b** with the vicinal and geminal proton peaks. For the major rotamer, coupling was observed between the proton (CH) at 4.92 ppm, the NH at 5.65 ppm, and the prochiral protons at 2.06 and 1.87 ppm. For the minor rotamer, the corresponding proton at 4.72 ppm coupled with the NH at 5.55 ppm and with the prochiral protons at 1.87 and 1.84 ppm.

The NOESY experiment (see Figure 4 in Supporting Information) confirmed the existence of two rotameric forms by showing exchange peaks between protons of both populations and transfer peaks between spacially near protons in the same species. For example, the N-methyl group from major species at 3.19 ppm gave NOE transfer peaks with a proton at 4.92 ppm and S-methyl at 2.11 ppm and gave transfer peaks with proton at 4.73 ppm and S-methyl at 2.07 ppm. The two different rotameric species are designated in Figure 5.

Literature methods for coupling N-alkylated amino acids (Scheme 8) have used (i) the usual carbodiimide based coupling reagents, BOP,[41] (ii) CF_3NO_2-PyBrOP,[42] (iii) BEP and FEP,[43] (iv) PyBroP,[42] (v) active ester,[44] and (vi) acid chloride method followed by N-methylation with diazomethane.[45] Yields of 62−

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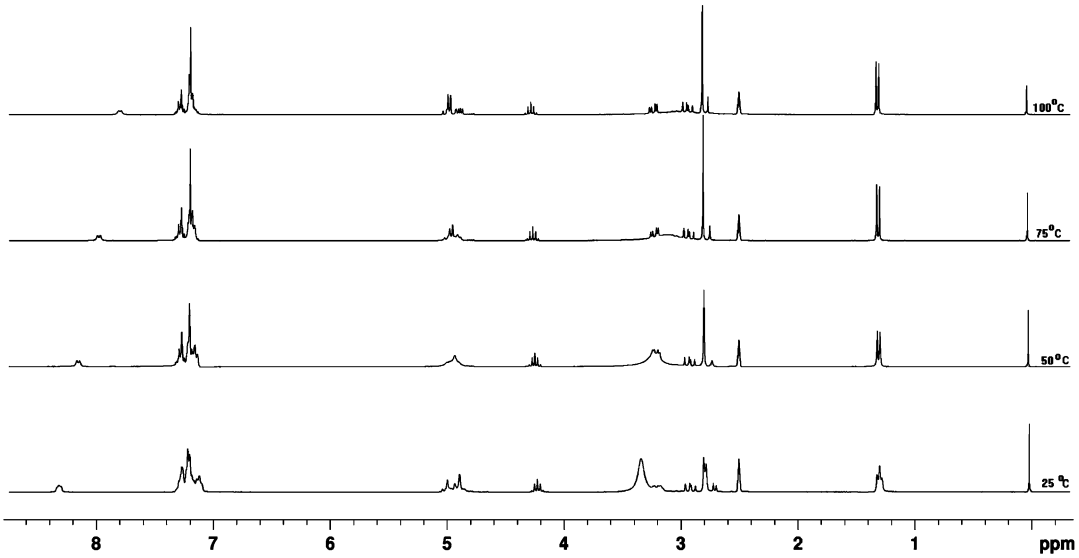
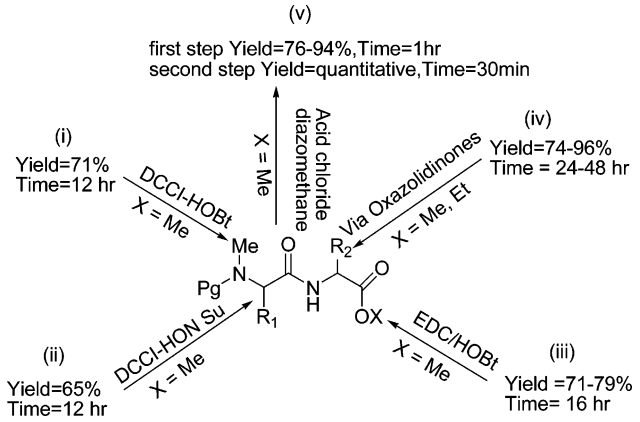


FIGURE 2. Variable temperature ^{1}H NMR of compound **6b.**

SCHEME 6. Literature Peptide Coupling Procedures Involving Activated Carboxyl Groups of *N*-methyl Amino Acids



SCHEME 7

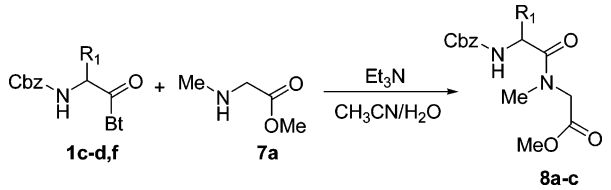


TABLE 4. Preparation of Dipeptides 8a−c from *N*-(Cbz-α-aminoacyl)benzotriazoles 1c−d,f and *N*(Me)-Gly-OMe 7a

			yield (%)[a]	
entry	reactant	product	20 °C[b]	MW[c]
1	Cbz-L-Phe-Bt **1c**	Cbz-L-Phe-*N*(Me)-Gly-OMe **8a**[c]	57	75
2	Cbz-L-Met-Bt **1d**	Cbz-L-Met-*N*(Me)-Gly-OMe **8b**[d]	51	72
3	Cbz-Gly-Bt **1f**	Cbz-Gly-*N*(Me)-Gly-OMe **8c**	66	80

[a] Isolated yield. [b] Power 200 W for 30 min at 55 °C and 300 W for 30 min at 60 °C. [c] HPLC for **8a**: 6.17 min. [d] HPLC for **8b**: 6.23 min.

99% and reaction times of 1−16 h were reported depending upon the Pg and −R group incorporated. Our methodology led to optically pure dipeptides (75−80%), is insensitive to water, and uses simple preparative and workup procedures and short reaction times (1 h).

III. Preparation of Proline-Containing Dipeptides. (a) Using *N*-Cbz-proline Acyl Bt. L-Amino acids **2a−d,f** were coupled with Cbz-Pro-Bt **1h**[22] to give products **9a−e** in yields of 70−95% (Scheme 9 and Table 5); the procedure was similar to that utilized above for the preparation of **6a−c** (Scheme 5).

At 20 °C, the ^{1}H and ^{13}C NMR spectra of **9c−e** were complex. However, the ^{1}H NMR spectra at 100 °C for **9c−e** were simplified, demonstrating the existence of rotamers, and confirming the absence of racemization (for the example of **9c**, see Figure 6). HPLC analysis revealed single retention times for **9a−e**, supporting their enantiopurity (Table 5).

Previously, proline peptides were prepared (Scheme 10) in reaction times of 24−48 h via (i) carbodiimide,[46] (ii) DCCI,[47] (iii) EDCI, HOBt,[48] or (iv) active ester methods using *p*-nitrophenyl, alkyl chloroformate, or *N*-hydroxysuccinimide.[47,49] in yields up to 85% for those reported depending upon the Pg and −R group incorporated. Our methodology led to optically pure dipeptides in yields of 70−95% using simple preparative and workup procedures and reaction times of 1 h.

(b) By Coupling at the NH Group of Free Proline. *N*-(Cbz-α-aminoacyl)benzotriazoles **1c,e,f,i** and **(1c + 1c′)** were coupled with L-Pro **2g** in partially aqueous solution (CH_3CN/H_2O) in the presence of Et_3N for 1 h at room temperature to give dipeptides **10a−d** and **(10a + 10a′)** in 63−89% yields in high purity (Scheme 11 and Table 6). NMR analysis of the enantiopure LL-dipeptides **10a−d** showed no detectable racemization (<5%). The enantiopurity of dipeptides **10a−d** was confirmed by HPLC analyses using Chirobiotic T column (detection at 254 nm, flow rate 0.5 mL/min, and MeOH as solvent): dipeptides **10a,d** each gave a single retention time, whereas the

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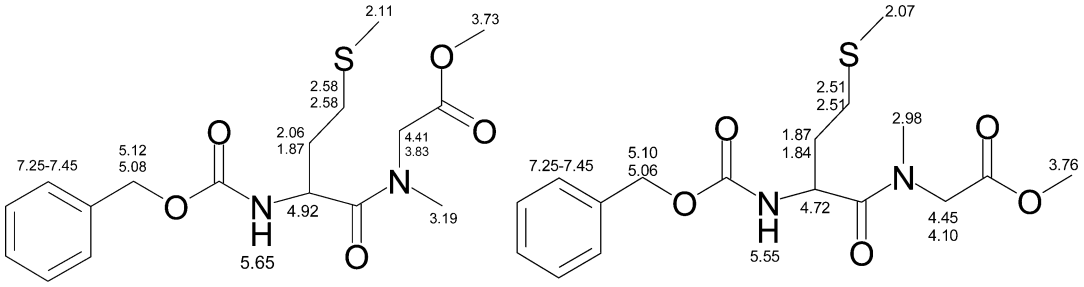
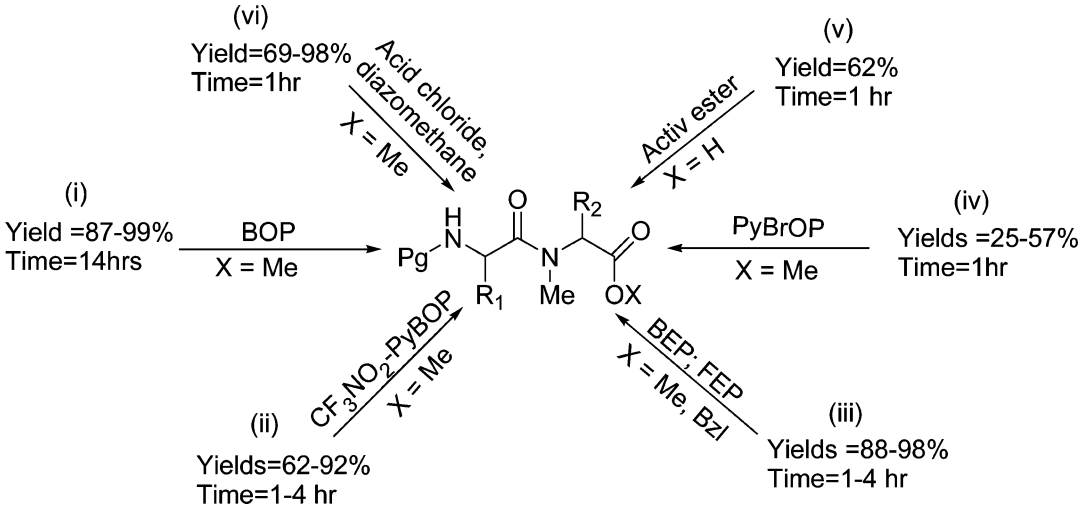
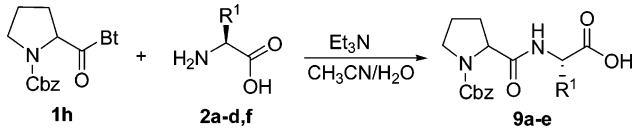


FIGURE 5. Two rotameric forms of compound **8b**.

SCHEME 8. Literature Peptide Coupling Procedures with Amino Group of *N*-Methyl Amino Acids



SCHEME 9



1h **2a-d,f** **9a-e**

TABLE 5. Preparation of Dipeptides 9a−e from *N*-Cbz-L-Pro-Bt 1h and Free Amino Acids 2a−d,f

entry	reactant	product	yield (%)[a]	retention time (min)
1	L-Phe-OH **2a**	Cbz-L-Pro-L-Phe-OH **9a**	91	7.13
2	L-Trp-OH **2b**	Cbz-L-Pro-L-Trp-OH **9b**	78	7.14
3	L-Met-OH **2c**	Cbz-L-Pro-L-Met-OH **9c**	82	7.48
4	L-Ala-OH **2d**	Cbz-L-Pro-L-Ala-OH **9d**	70	7.16
5	L-Val-OH **2f**	Cbz-L-Pro-L-Val-OH **9e**	95	7.14

[a] Isolated yield.

diastereomeric mixture **(10a + 10a′)** showed two retention times as expected (Table 6).

Previous preparations of proline peptides by coupling at the proline NH were achieved via carbodiimide methods[47,50,51] (i−iii) and active ester methods[47,52,53] (iv−vi) (Scheme 12). Our method synthesizes proline peptides in short reaction times utilizing mild reaction conditions.

Conclusions

The methodology described in the present paper provides for the convenient and efficient preparation of hindered peptides in short reaction times utilizing simple workup procedures. It is noteworthy that the reactivity of a sterically hindered amino acid differs depending on whether the N- or the C-terminus is undergoing coupling. Coupling using C-activated hindered amino acids generally proceeds smoothly in high yields under mild aqueous reaction conditions. In contrast, coupling at a hindered NH group under similar conditions results in hydrolysis of the (aminoacyl)Bt component. This behavior is probably due to competitive hydrolysis; that is, hydrolysis is more important when the steric strain is close to the NH site and hinders the coupling.

Experimental Section

General Procedure for the Preparation of Dipeptides 3a−e, (3c + 3c′), 6a−c, (6b + 6b′), 9a−e, 10a−d, (10a + 10a′). *N*-(Protected-α-aminoacyl)benzotriazoles (1 mmol) were added at 20 °C to a solution of α-amino acid (1 mmol) in CH_3CN (7 mL)/H_2O (3 mL) in the presence of Et_3N (2 mmol). The reaction mixture was stirred at 20 °C until the starting material was completely consumed as observed by TLC using hexanes/ethyl acetate (2:1) as the solvent. After 1 mL of 4 N HCl was added, the solution was concentrated under reduced pressure to remove acetonitrile. The solution was extracted with EtOAc (20 mL), and the organic extract was washed with 4 N HCl (3 × 5 mL) and saturated NaCl (10 mL) and then dried (anhydrous $MgSO_4$). Evaporation of the solvent

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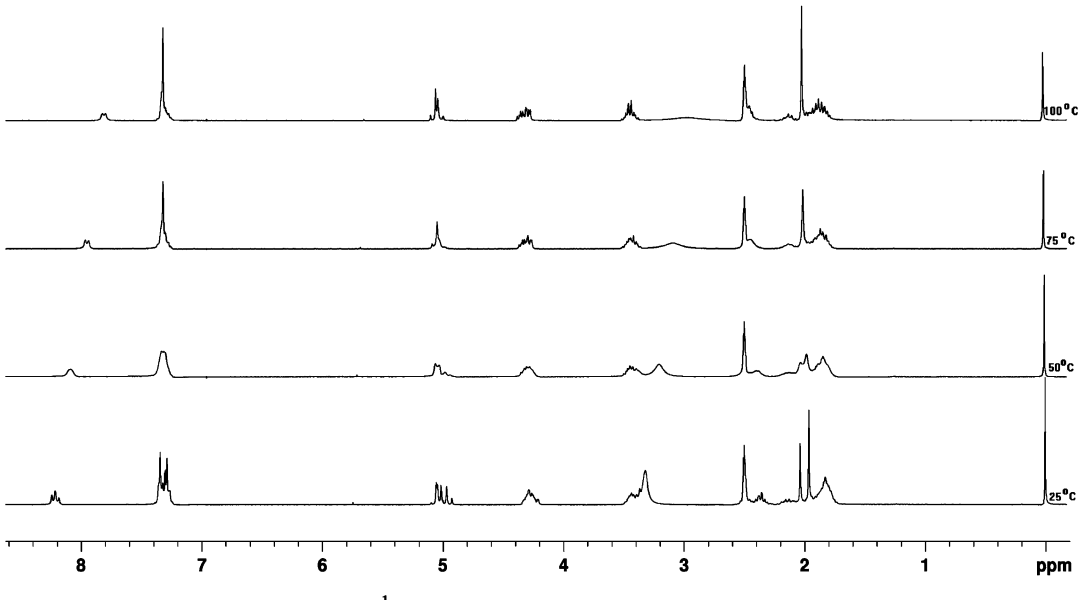
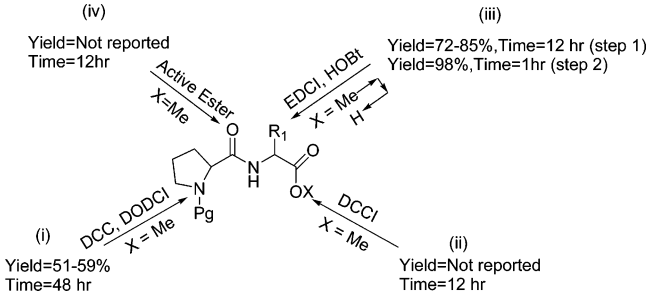


FIGURE 6. Variable temperature ^{1}H NMR of compound **9c.**

SCHEME 10. Literature Peptide Coupling Procedures with Activated Carboxyl Group of Proline



SCHEME 11

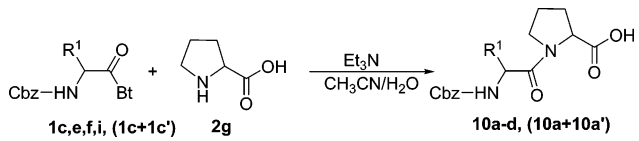


TABLE 6. Preparation of Dipeptides 10a−d and Diastereomeric Mixture (10a + 10a′) from *N*-(Cbz-α-aminoacyl)benzotriazoles 1c,e,f,i, (1c + 1c′) and L-Pro 2g

entry	reactant	product	yield (%)[a]
1	Cbz-L-Phe-Bt **1c**	Cbz-L-Phe-L-Pro-OH **10a**[b]	81
2	Cbz-DL-Phe-Bt (**1c** + **1c**)	Cbz-DL-Phe-L-Pro-OH (**10a** + **10a′**)[c]	90
3	Cbz-L-Trp-Bt **1e**	Cbz-L-Trp-L-Pro-OH **10b**	89
4	Cbz-Gly-Bt **1f**	Cbz-Gly-L-Pro-OH **10c**	74
5	Cbz-L-Ala-Bt **1i**	Cbz-L-Ala-L-Pro-OH **10d**[d]	63

[a] Isolated yield. [b] HPLC result for **10a**: 6.20 min. [c] HPLC for **(10a + 10a′)**: 6.10 and 7.09 min. [d] HPLC for **10d**: 7.16 min.

gave the desired product in pure form, which was further recrystallized from CH_2Cl_2/hexanes unless otherwise specified.

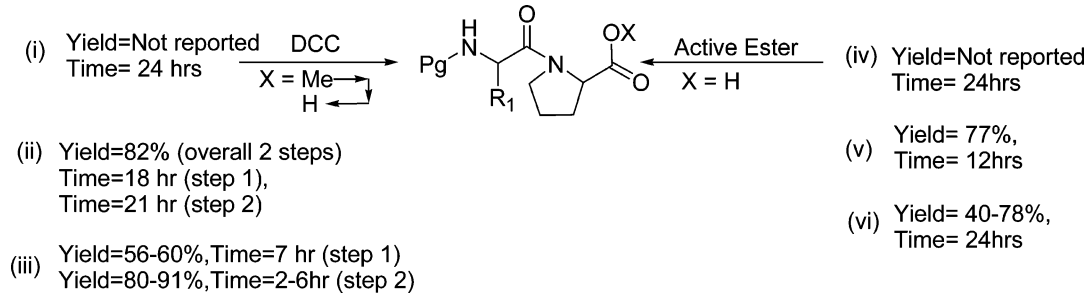
(2*S*)-2-[(2-[(Benzyloxy)carbonyl]amino-2-methylpropanoyl)-amino]-3-(1*H*-indol-3-yl)propanoic Acid (Cbz-Aib-L-Trp-OH, 3b). Colorless microcrystals (85%). mp 75−77 °C. $[\alpha]_D^{23} = +13.2$ (*c* 2.16, DMF). ^{1}H NMR (DMSO-d_6): δ 1.30 (s, 3H), 1.32 (s, 3H), 3.13 (J = 6.6 Hz, 2H), 4.47 (q, J = 6.9 Hz, 1H), 4.93 (d, J = 12.6 Hz, 1H), 5.00 (d, J = 12.6 Hz, 1H), 6.96 (br t, J = 7.2 Hz, 1H), 7.05 (br t, J = 7.2 Hz, 1H), 7.10 (d, J = 1.8 Hz, 1H), 7.26−7.38 (m, 7H), 7.45 (d, J = 7.8 Hz, 1H), 7.51 (d, J = 7.8 Hz, 1H), 10.87 (s, 1H), 12.67 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 24.8, 25.2, 26.9, 52.8, 55.9, 65.1, 109.5, 111.2, 118.1, 118.2, 120.8, 123.5, 127.2, 127.6, 128.2, 136.0, 136.8, 154.6, 173.1, 173.9. Anal.Calcd for $C_{23}H_{25}N_3O_5$: C, 65.24; H, 5.95; N, 9.92. Found: C, 64.88; H, 6.12; N, 9.67.

(2*S*)-2-((2*S*)-2-[[(Benzyloxy)carbonyl](methyl)amino]-3-phenylpropanoylamino)propanoic Acid (Cbz-L-*N*(Me)-Phe-L-Ala-OH, 6b). Recrystallized from diethyl ether/hexanes to give colorless microcrystals (72%). mp 89−91 °C. $[\alpha]_D^{23} = -4.6$ (*c* 1.90, DMF). ^{1}H NMR (DMSO-d_6, 100 °C): δ 1.31 (d, J = 7.3 Hz, 3H), 2.82 (s, 3H), 2.95 (dd, J = 14.7, 10.6 Hz, 1H), 3.24 (dd, J = 14.6, 5.1 Hz, 1H), 4.30 (q, J = 7.3 Hz, 1H), 4.91 (dd, J = 10.6, 5.1 Hz, 1H), 4.97 (d, J = 12.5 Hz, 1H), 5.03 (d, J = 12.5 Hz, 1H), 7.23−7.35 (m, 10H), 7.84 (d, J = 6.7 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 °C): δ 16.6, 30.2, 34.0, 47.2, 59.2, 65.8, 125.6, 126.6, 127.0, 127.5, 127.7, 128.2, 136.4, 137.5, 155.3, 169.2, 172.9. Anal. Calcd for $C_{21}H_{24}N_2O_5$: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.89; H, 6.33; N, 7.01.

General Procedure for the Preparation of Dipeptides 5a−d and (5a + 5a′). Method A. *N*-(Protected-α-aminoacyl)benzotriazoles (1 mmol) were added at 20 °C to a solution of α-amino acid esters (1 mmol) in CH_3CN (7 mL) in the presence of Et_3N (2 mmol). The reaction mixture was then stirred at 20 °C until the starting material was completely consumed as observed by TLC using hexanes/ethyl acetate (2:1) as the solvent. The solution was concentrated under reduced pressure to remove acetonitrile. The solution was extracted with EtOAc (20 mL), and the organic extract was washed with saturated Na_2CO_3 (3 × 5 mL) and saturated NaCl (10 mL) and then dried (anhydrous $MgSO_4$). Evaporation of the solvent gave the desired product in pure form, which was further recrystallized from CH_2Cl_2/hexanes unless otherwise specified.

Method B. A mixture of *N*-(protected-α-aminoacyl)benzotriazoles (1 mmol), α-amino acid esters (1 mmol), and Et_3N (2 mmol) in THF (7 mL) was stirred in a sealed tube (10 mL) under microwave irradiation (200 W) at 55 °C for 30 min and an additional 30 min at 60 °C (300 W). The solution was concentrated under reduced pressure to remove THF, and the concentrate was extracted with EtOAc (20 mL). The organic extract was washed with saturated Na_2CO_3 (3 × 5 mL) and saturated NaCl (10 mL) and then dried (anhydrous $MgSO_4$). Evaporation of the solvent gave

SCHEME 12. Peptide Coupling with N-Terminus Proline



the desired product in pure form, which was further recrystallized from CH_2Cl_2/hexanes unless otherwise specified

Methyl 2-[((2*S*)-2-[(Benzyloxy)carbonyl]amino-3-phenylpropanoyl)amino]-2-methylpropanoate (Cbz-L-Phe-Aib-OMe, 5a). Recrystallized from EtOAc/hexanes to give colorless microcrystal needles (method A 41%, method B 72%). mp 105−107 °C, lit.[33] mp 88−90 °C. $[\alpha]_D^{23} = -10.2$ (*c* 1.05, DMF). ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 3H), 1.43 (s, 3H), 2.98 (dd, J = 13.7, 8.0 Hz, 1H), 3.14 (dd, J = 13.7, 6.0 Hz, 1H), 3.70 (s, 3H), 4.30−4.42 (m, 1H), 5.10 (s, 2H), 5.36 (d, J = 7.1 Hz, 1H), 6.11 (s, 1H), 7.19−7.38 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 24.4, 24.5, 38.6, 52.7, 56.2, 56.5, 67.0, 127.1, 128.0, 128.2, 128.5, 128.7, 129.5, 136.3, 136.4, 155.9, 169.7, 174.5. Anal. Calcd for $C_{22}H_{26}N_2O_5$: C, 66.32; H, 6.58; N, 7.03. Found: C, 66.36; H, 6.85; N, 7.24.

General Procedure for the Preparation of Dipeptides (8a−c). Compounds **8a−c** were prepared according to the general procedure for preparation of compounds **5a−d** (method A and method B using 4 mmol of Et_3N instead of 2 mmol.

Methyl 2-[[(2*S*)-2-[(Benzyloxy)carbonyl]amino-4-(methylsulfanyl)butanoyl](methyl)amino]acetate (Cbz-L-Met-*N*(Me)-Gly-OMe, 8b). Oil (method A 51%, method B 72%). lit.[14] mp not reported. $[\alpha]_D^{23} = -10.9$ (*c* 1.83, DMF). ^{1}H NMR ($CDCl_3$, method A): δ 1.84−1.95 (m, 1.5H), 1.99−2.06 (m, 0.5H), 2.08 (s, 0.5H), 2.11 (s, 2.5H), 2.48−2.63 (m, 2H), 2.95 (s, 0.5H), 3.19 (s, 2.5H), 3.74 (s, 2.5H), 3.77 (s, 0.5H), 3.84 (d, J = 16.8 Hz, 0.2H), 4.11 (d, J = 18.0 Hz, 0.8H), 4.42 (d, J = 16.0 Hz, 0.8H), 4.46 (d, J = 18.1 Hz, 0.2H), 4.67−4.77 (m, 0.2H), 4.88−4.98 (m, 0.8H), 5.10 (br s, 2H), 5.56 (d, J = 8.7 Hz, 0.2H), 5.66 (d, J = 8.7 Hz, 0.8H), 7.30−7.38 (m, 5H). ^{13}C NMR ($CDCl_3$, method A, two rotameric forms): δ 15.4, 15.6, 29.8, 31.5, 32.2, 36.6, 49.8, 53.0, 67.1, 67.2, 128.0, 128.2, 128.3, 128.5, 128.6, 136.0, 136.2, 156.1, 156.2, 172.4, 173.0, 175.7. Anal. Calcd for $C_{17}H_{24}N_2O_5S$: C, 55.42; H, 6.57; N, 7.60. Found: C, 55.11; H, 6.81; N, 7.51.

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Supporting Information Available: Compound characterization data for **1a,b,g**; **3a,c−e, (3c + 3c′)**; **(5a + 5a′)**, **5b−d**; **6a, c**, **(6b + 6b′); 8a,c**; **9a−e;** and **10a−d**, **(10a + 10a′)** is available free of charge via the Internet at http://pubs.acs.org.

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